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Review

ω-Amino Acid : Pyruvate Aminotransferase

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KEY WORDS: ω-amino acid: pyruvate aminotransferase / γ-aminobutyrate transaminase / *Pseudomonas* / gabaculine / stereochemistry / X-ray crystallography / enzymatic assay /

Abbreviations used: pyridoxal-P (PLP); pyridoxal 5′-phosphate, pyridoxamine-P (PMP); pyridoxamine 5′-phosphate, pyridoxine-P; pyridoxine 5′-phosphate, MBTH; 3-methyl-2-benzothiazolone hydrazone hydrochloride, DTNB; 5, 5′-dithio-bis(2-nitrobenzoic acid).

INTRODUCTION

Among the many pyridoxal-P dependent transaminases as far known, two categories can be constructed: those which carry out chemistry at the α-carbon of a susceptible α-amino acid and those which oxidatively deaminate the ω-amino group of an ω-amino acid. As reviewed by Braunstein, many enzymologic studies employing the purified enzyme have been performed on α-amino acid transaminase.[1] ω-Amino acids occur extensively in animal tissues, plants and microorganisms as a free or a conjugated form.[2] It is now well established that γ-aminobutyrate is a major inhibitory transmitter in many invertebrate systems and in the vertebrate central nervous system.[3~5] In addition the compound is suggested to be important for the germination of bacterial spore.[6] β-Alanine is a consistuent of coenzyme A and a precursor of pantothenic acid. 2-Aminoethane sulfonate (taurine, an ω-amino sulfonic acid) is one of the most abundant amino acids in mammalian tissues, and many studies are actively progressing to elucidate its physiological function.[7] Transamination is important for the formation and degradation of these ω-amino acids and is considered to be the first setp of catabolism of these compounds. Accordingly it has become increasingly important to obtain detailed knowledge about the ω-amino acid transaminase. There have been two enzymes mainly involved in the transamination of ω-amino acid *i.e.* β-alanine and γ-aminobutyrate transaminases which catalyze the following reaction. In general,

$$\beta\text{-Alanine} + \text{pyruvate} \rightleftharpoons \text{Malonic semialdehyde} + \text{L-Alanine}$$

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γ-Aminobutyrate + α-Ketoglutarate ⇌ Succinic semialdehyde + L-Glutamate

β-alanine transaminase is characterized by its requirement for pyruvate and γ-aminobutyrate transaminase for α-ketoglutarate as an amino acceptor. With the latter enzyme, many studies have been carried out using mammalian enzyme with an emphasis on its physiological function.[8~10] Some of them were purified to homogeneity and partially characterized. There are another ω-amino acid transaminase catalyzing transamination of ω-amino group of α, ω-diaminocarboxylic acid such as C_6 of L-lysine and C_5 of L-ornithine, which were purified and crystallized from bacteria. These enzymes, however, do not catalyze the transamination of γ-aminobutyrate or β-alanine. Taurine : α-ketoglutarate aminotransferase catalyzing transamination of ω-amino acids besides that of taurine was also purified and crystallized from *Achromobacter superficialis*.[13] Recently, we purified and crystallized ω-amino acid: pyruvate and γ-aminobutyrate: α-ketoglutarate aminotransferase from *Pseudomonas* sp. F–126, which enabled us to characterize the enzymologic properties in detail.[14~18] In this review we will try briefly to present and discuss some properties of the ω-amino acid transaminase in comparison with other transaminases, especially γ-aminobutyrate transaminase. ω-Amino acid : pyruvate aminotransferase designated previously taurine : pyruvate aminotransferase[14] can be classified as β-alanine : pyruvate aminotransferase (E.C. 2.6.1.18). The terminology, however, of "ω-amino acid: pyruvate aminotransferase" is more relevant from the view point of its substrate specificity as described below.

OCCURRENCE

ω-Amino acid : pyruvate aminotransferase was found in bacteria[14, 19, 20] and plants,[21] but not in mammalian tissues, where an enzyme catalyzing transamination between β-aminoisobutyrate and pyruvate was demonstrated.[22] The enzyme also catalyzes the transamination between β-alanine and pyruvate. On the contrary, γ-aminobutyrate transaminase was found extensively in many ammmalian tissues,[3~5] bacteria[23, 24] and plants.[25] In bacteria, the enzyme activity is constitutive to some extent enhanced by γ-aminobutyrate in the growth medium, though ω-amino acid: pyruvate aminotransferase is induced by β-alanine. *Pseudomonas* sp. F–126 and *Bacillus cereus*[20] produced the two ω-amino acid transaminases.

PURIFICATION

ω-Amino acid : pyruvate aminotransferase was purified from *Pseudomonas* sp. F–126 isolated from soil in the medium containing taurine as the single carbon and nitrogen source (Table I).[14, 15] The purified enzyme was homogeneous on the criteria of Disc gel electrophoresis and ultracentrifugation (Fig. 1). By adding solid ammonium sulfate to the enzyme solution, the enzyme was crystallized. The crystal took a form of rod shape (Fig. 2). In Fig. 2, the crystals of γ-aminobutyrate : α-ketoglutarate aminotransferase purified from the same organism (Table II) is also presented. Table

Table I. Purification of ω-Amino Acid: Pyruvate Aminotransferase

Step	T. protein (mg)	T. activity (units)	S. activity (units/mg)	Yield (%)
Sonicate	140,000	9,800	0.07	100
Polyethyleneimine	102,025	7,243	0.07	78
First DEAE-cellulose	6,205	5,026	0.81	53
Second DEAE-cellulose	2,760	4,305	1.56	46
Hydroxylapatite	992	3,204	3.23	33
Sephadex G-150	736	2,590	3.52	28

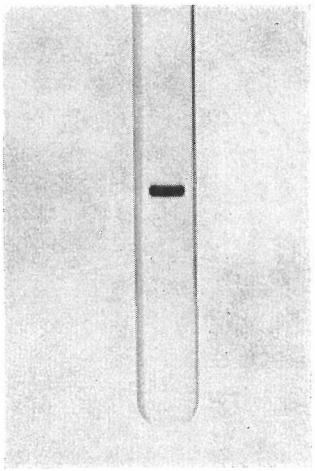
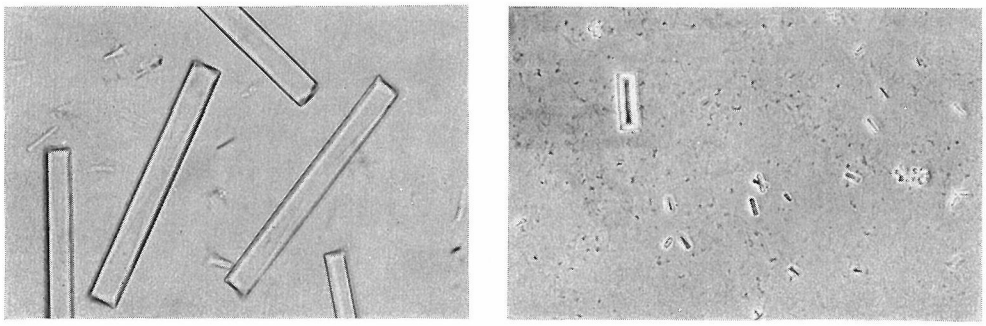


Fig. 1. Disc-gel electrophoresis.



(A) (B)

Fig. 2. Crystals of ω-amino acid: pyruvate aminotransferase (A) and γ-aminobutyrate: α-keto-glutarate aminotransferase (B).

III gives the relative activities for β-alanine and γ-aminobutyrate of the purified or partially purified enzyme including γ-aminobutyrate transaminase.

Table II. Purification of γ-Aminobutyrate: α-Ketoglutarate Aminotransferase

Step	Protein (mg)	Activity (units)	Specific activity (units/mg)	Yield (%)
Sonicate	26,510	9,522	0.36	100
Polyethyleneimine	25,740	9,306	0.36	97
First DEAE-cellulose	4,100	9,108	2.16	96
First hydroxylapatite	1,400	4,680	3.24	49
Sephacryl S-200	465	4,050	8.46	42
Second DEAE-cellulose	150	3,456	21.78	34
Second hydroxylapatite	81	2,880	35.46	30
Third DEAE-cellulose	45	2,106	46.80	22

Table III. Substrate Specificity of β-Alanine and γ-Aminobutyrate Transaminae

	Amino acceptor		Amino donor		Reference
	Pyruvate	α-KGA*	β-Ala*	γ-ABA*	
	(Relative activity)				
β-Alanine transaminase					
Pseudomonas sp. F-126[a]	100	0	100	40	14
P. fluorescens[b]	100	0	1000	118	19
Bacillus cereus[b]	100	31	100	43	20
γ-Aminobutyrate transaminase					
Pseudomonas sp. F-126[a]	0	100	0	100	26
P. aeruginosa[a]	ND	100	0.5	100	25
B. cereus[b]	6	100	3	100	20
Mouse brain[a]	0	100	100	100	8
Pig liver[a]	ND	100	100	100	10
Pig brain[a]	ND	100	126	100	27

* α-KGA: α-Ketoglutarate, β-Ala: β-Alanine, γ-ABA: γ-Aminobutyrate

a) Purified enzyme, b) Partially purified enzyme

ND: Not determined

MOLECULAR WEIGHT AND SUBUNIT STRUCTURE

The molecular weight of ω-amino acid: pyruvate aminotransferase was determined to be about 172,000 by gel-filtration and ultracentrifugation methods.[15,16] The enzyme migrated as a single polypeptide with a molecular weight of 44,000 in a Disc gel electrophoresis containing SDS, suggesting that the enzyme is composed of four subunits identical in molecular weight. The identity of subunits was also confirmed by X-ray crystallographic analysis as discussed below. γ-Aminobutyrate transaminase of *Pseudomonas* sp. F-126 has the molecular weight of 176,000.[26] In general, bacterial ω-aminoacid transaminases have molecular weights ranging between 160,000 and 180,000, and while the mammalin enzymes range between 100,000 and 120,000 daltons. This suggests that the bacterial enzyme is composed of four subunits and the mammalin enzyme has two subunits.

ENZYMOLOGIC PROPERTIES

1) Effect of pH and temperature[16]

ω-Amino acid: pyruvate aminotransferase shows optimum pH for the reaction in alkaline pH and is stable between pH 7.0–10.0 (Fig. 3). These are a common pH profile for ω-amino acid transaminases.

The transaminase reaction was affected by temperatures. The activity was linearly enhanced with an increase in temperature. The maximum activity was obtained at around 60°C (Fig. 4A), and the enzyme was stable at temperatures up to 65°C (Fig. 4B). The activity was increased to some extent by incubation with pyridoxal-P at 40–60°C

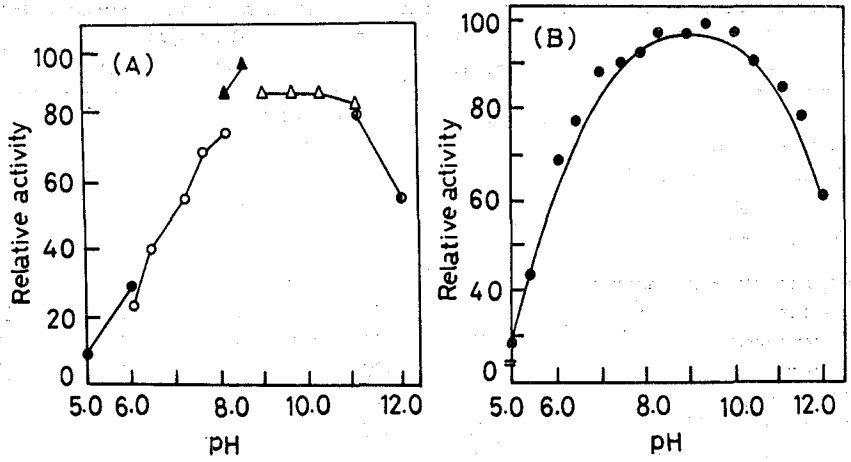


Fig. 3. Effect of pH on activity (A) and stability (B) of ω-amino acid: pyruvate aminotransferase.

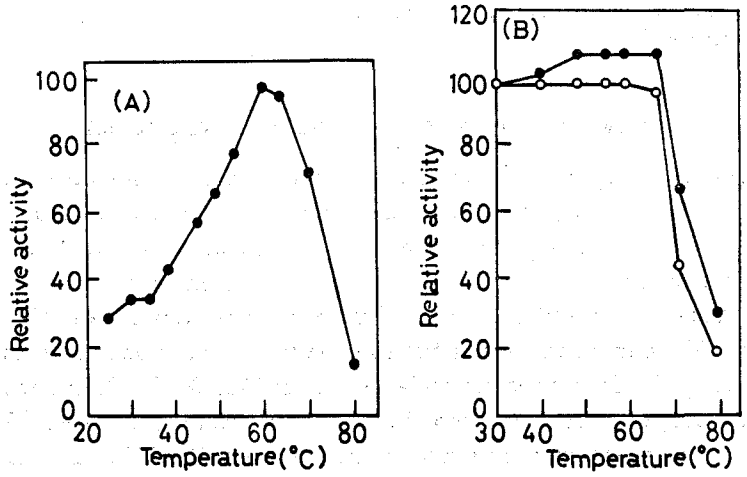


Fig. 4. Effect of temperature on activity (A) and stability (B) of ω-amino acid: pyruvate aminotransferase.

("Heat-activation") but not at a physiological temperature. In addition the compound protected the enzyme against thermal inactivation. "Heat-activation" in the presence of pyridoxal-P was observed for other ω-amino acid transaminase. Taurine transaminase of *Achromobacter superficialis* was purified in the catalytically inactive form and reactivated fully by "Heat-activation."[13] L-Lysine ε-aminotransferase was also activated by incubation with pyridoxal-P at 60°C.[11] Although γ-aminobutyrate transaminase of *Pseudomonas* sp. F-126 did not show "Heat-activation", the enzyme activity was protected by the presence of pyridoxal-P against thermal inactivation.[26] The properties, "Heat-activation" and/or the protection against thermal inactivation by the cofactor, have not yet been studied for a mammalian ω-amino acid transaminase. Of the α-amino group transaminase only tyrosine transaminase was reported for "Heat-activation". ω-Amino acid: pyruvate aminotransferase inactivated by carbonyl reagents was reactivated by pyridoxal-P more effectively at 60°C than at 37°C (Table IV). Pyridoxamine-P was as effective activator as pyridoxal-P, but either pyridoxine,

Table IV. Reactivation of the Carbonyl Reagents-Inhibited Enzyme by Pyridoxal-P and Pyridoxamine-P

Compounds (0.5 mM)	None	+PLP		+PMP	
		37°C	60°C	37°C	60°C
None	100	101	110	99	110
Phenylhydrazine	9	31	54	24	39
MBTH*	7	70	105	53	92
D-Cycloserine	13	33	79	27	60
Aminoxyacetate	4	15	18	11	13

* MBTH: 3-methyl-2-benzothiazolone hydrazone hydrochloride

pyridoxal, pyridoxamine or pyridoxine-P could not reactivate the enzyme. These results indicate that the reactivation is not ascribed to promotion of the formation of a Schiff base between pyridoxal-P and ε-amino group of lysine residue of the enzyme protein, but rather to a conformation change to facilitate a binding of the cofactor to the active site. In confirmity with this view the following explanation for "Heat-activation" and the protection against thermal inactivation is conceivable that the enzyme is partially resorved from the cofactor to a unstable form and a conformational change caused by heating with the cofactor stabilizes the active form of the enzyme. The other explanation with less probability is that the cofactor binds to a site other than the active site to activate and stabilize the enzyme with a concomitant conformation change of the enzyme protein. Although more detailed studies are required to elucidate a mechanism of the activation and the protection, these properties seem to be a common characteristic of ω-amino acid aminotransferase.

2) Inhibitors[16]

Pyridoxal-P dependent enzymes are inhibited by carbonyl reagents such as phenylhydrazine, aminoxyacetate, hydroxylamine and D-cycloserine. These compounds

also inhibited ω-amino acid: pyruvate aminotransferase activity. Table V shows the effect of various inhibitors on the ω-amino acid transaminase. Most of carbonyl reagents inhibited the activity, but not sulf hydryl reagents or chelating agents.

Table V. Effect of Inhibitors on the ω-Amino Acid: Pyruvate Aminotransferase

Compounds (0.5 mM)	Relative activity
None	100
Phenylhydrazine	6
MBTH	4
D-Penicillamine	65
L-Penicillamine	60
D-Cycloserine	8
Hydroxylamine	0
D-Cysteine	87
L-Cysteine	85
Aminoxyacetate	2

No effect: $HgCl_2$, *p*-chloromercuribenzoate, monoiodoacetate, 5, 5′-dithio-bis(2-nitrobenzoic acid), EDTA, diethylthiocarbamate, tiron, 8-oxyquinoline, 0-phenanthroline, α, α′-dipyridyl, L-aspartate, L-phenylalanine, L-methionine, L-valine, L-glutamate, L-serine, L-lysine, L-ornthine.

The enzyme inactivated by phenylhydrazine, MBTH, D-cycloserine and aminoxyacetate were reactivated by pyridoxal-P or pyridoxamine-P (Table IV). The reactivation was more effective at 60°C than at 37°C. Based on these results, it was suggested that the inactivated cofactor with carbonyl reagents could be replaced by the cofactors to generate the active enzyme and this process was accompanied with a conformational change of the enzyme protein as mentioned before.

Although a sulf hydryl group is essential for the catalytic activity of various transaminases including ω-amino acid transaminase,[1] the activity of ω-amino acid: pyruvate aminotransferase is not affected by thiol reagents, suggesting that the thiol group of the enzyme may not play an essential role in the catalytic action or is burried in a region of the enzyme which is inaccesible to sulf hydryl reagents. In contrast to the ω-amino acid transaminase, γ-aminobutyrate transaminase from the same organism (*Pseudomonas* sp. F–126) was completely inhibited with sulf hydryl reagents (Table VI).[26] The other ω-amino acid transaminase including β-alanine : pyruvate aminotransferase from *P. fluorescens* were also inhibited by the reagents. ω-Amino acid : pyruvate aminotransferase is unique in this regard.

3) Substrate specificity[16~18]

ω-Amino acid : pyruvate aminotransferase catalyzes transamination between various ω-amino acids and pyruvate as shown in Table VII. 2-Aminoethan sulfinate (hypotaurine), 3-aminopropane sulfonate, β-alanine, γ-aminobutyrate, 7-amino-

Table VI. Effect of Inhibitors on γ-Aminobutyrate: α-Ketoglutarate Aminotransferase

Compounds	Relative activity
None	100
NH_2OH	0
Aminooxyacetate	0
D-Penicillamine	27
D-Cycloserine	12
Phenylhydrazine	15
MBTH	10
L-Cysteine	0
D-Cysteine	0
DTNB	25
N-Ethylmaleimide	0
p-Chloromercuribenzoate	0
$HgCl_2$	0
Gabaculine	0
$CuCl_2$	32

No effect: monoiodoacetate, L-methionine, L-phenylalanine, taurine, D-serine, L-serine, L-aspartate, D-aspartate, L-α-aminobutyrate, D-α-aminobutyrate, 2, 4-diaminobutyrate, $CaCl_2$, $ZnCl_2$, $CdCl_2$, $MnCl_2$, $CoCl_2$, $FeSo_4$, $BaCl_2$, $MgCl_2$, tiron, α, α′-dipyridyl, ethylenediaminetetraacetate, and *o*-phanthroline.

Table VII. Substrate Specificity for ω-Amino Acids

Amino donor	Relative activity
Taurine	3.6
Hypotaurine	132.0
Aminoethylhydrogen sulfate	1.5
Thiotaurine	0.7
3-Aminopropane sulfonate	80.0
Glycine	0.4
β-Alanine	100.0
γ-Amino-*n*-butyrate	40.0
δ-Aminovalerate	6.0
ε-Aminocaproate	8.2
7-Aminoheptanoate	35.0
8-Aminooctanoate	77.0
DL-β-Amino-*n*-butyrate	96.4
DL-β-Aminoisobutyrate	54.2

Inert: *p*-aminobenzoate, L-carnosine, aminomethane sulfonate, L-glutamate, L-aspartate, L-serine, L-α-amino-*n*-butyrate, L-phenylalanine, L-cysteine, L-ornithine, D- and L-lysine.

heptanoate, DL-3-amino-*n*-butyrate and DL-3-aminoisobutyrate are good amino donors, while 2-aminoethane sulfonate (taurine), 5-aminopentanoate and 6-aminohexanoate are not as active. As mentioned above the enzyme can be classified as β-alanine: pyruvate aminotransferase (E.C. 2.6.1.18). Various ω-amino acids, however, can serve as an amino donor more or less. Accordingly the terminology of ω-amino acid: pyruvate aminotransferase seem to be more relevant and is employed in this review. The kinetic pattern obtained either with varying concentration of β-alanine at several fixed concentration of pyruvate or with concentration of pyrnvate at fixed concentration of β-alanine showed a series of parallel line on a double reciprocal plot of velocity *vs.* substrate concentration (Fig. 5). This result indicates that transamination of

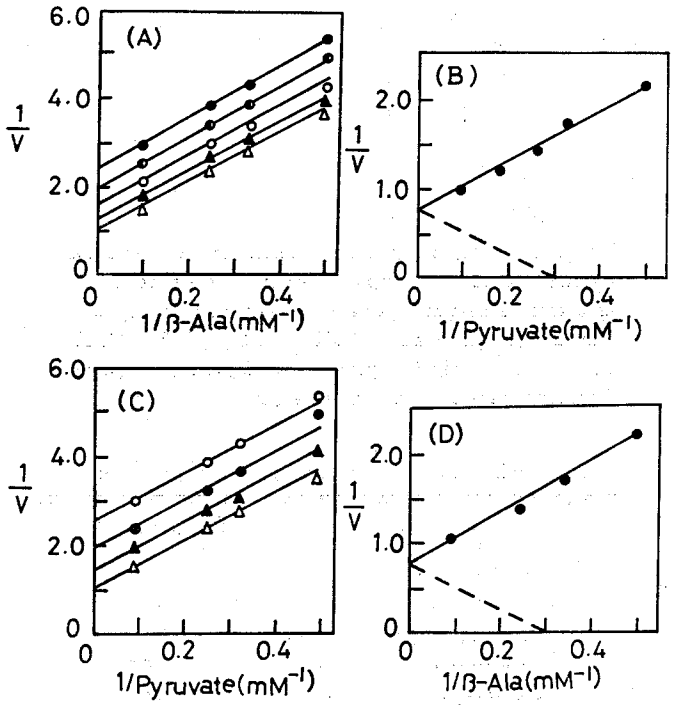


Fig. 5. Effect of concentrations of β-alanine and pyruvate on the enzyme activity.
—●—: 2.0 mM, ○: 3.0 mM, —■—: 4.0 mM (A)
—▲—: 4.0 mM (B), 5.0 mM (A), —△—: 10.0 mM

ω-aminoacid proceeds via ping-pong bi bi mechanism as observed for that of α-amino group. Michaelis constants for β-alanine and pyruvate are 3.3 mM. In addition to ω-amino acids, various mono- and diamines showed amino donor activity for the enzyme reaction (Table VIII and IX). The transamination also follows ping-pong bi bi mechanism, and Michaelis constants for *n*-butylamine and putrescine are 66.6 mM and 76.9 mM. In both reaction systems the constant for pyruvate are 5.5 mM and 6.25 mM, respectively. In contrast to broad substrate specificity for amino donor, the enzyme showed narrow substrate specificity for amino acceptor; pyruvate is an exclusive amino acceptor in both ω-amino acid and amine transaminations.

Table VIII. Amino Donor Specificity for Monoamines

Amino donor	Relative activity
β-Alanine	100
Methylamine	0
Ethylamine	30
Ethanolamine	5
n-Propylamine	35
iso-Propylamine	40
n-Butylamine	60
iso-Butylamine	50
sec-Butylamine	30
t-Butylamine	0
n-Amylamine	80
iso-Amylamine	30
t-Amylamine	0
n-Hexylamine	65
n-Heptylamine	60
n-Octylamine	45
Benzylamine	45
Phenetylamine	54
Tyramine	5
Tryptamine	20
Histamine	10

Table IX. Amino Donor Specificity for Diamines

Amino donor	Relative activity
β-Alanine	100
1 2-Diaminoethane	12
1, 3-Diaminopropane	0
1, 4-Diaminobutane	55
1, 5-Diaminopentane	38
1, 6-Diaminohexane	87
1, 7-Diaminoheptane	92
1, 8-Diaminooctane	25
Spermidine	0
Agmatine	0

Based on these results a separate binding site for amino donor (ω-site) and for pyruvate (and alanine, α-site) was suggested. ω-Amino acids and amines bind with ω-site, and pyruvate (and alanine) interacts with α-site. The enzyme activity was

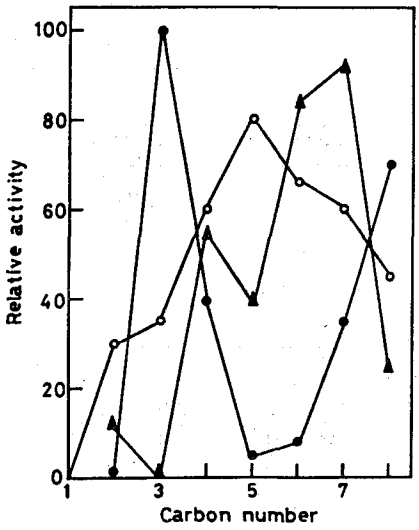


Fig. 6. Effect of length in carbon chain of ω-amino acid, alkylmono- and -diamines on the transamination.

—●—: ω-amino acids, —○—: monoamines, —▲—: diamines

affected by the length of the carbon chain of a straight-chain ω-aminocarboxylic acid, and alkylmono- and diamines (Fig. 6). Distinct patterns were observed between ω-amino acid and amine transamination. For the ω-amino acid transamination the enzyme was most active with a 3-carbon chain substrate (β-alanine) and then the activity was decreased with increasing carbon chain up to carbon atoms 6. With the carbon chain greater than 6 carbon atoms long, the enzyme activity increased. The mono- and diamine transaminase activities, on the whole, were similarly affected by the length of carbon chain with maxima activities at 5- and 7-carbon units, respectively. These results suggest that a distance between amino and carboxyl groups, and a hydrophobic interaction of the methylene with the ω-site are important for the enzymic transamination. A substitution of the 4th carbon atom of octamethylenediamine with nitrogen atom (*i.e.* spermidine) abolished the substrate activity of the compound, suggesting the importance of hydrophobic interaction. Of the diamine examined, 1, 3-diaminopropane was inert. In addition the compound inactivated the enzyme. Based on spectral data it was shown that the inactivation of the enzyme with 1, 3-diaminopropane was due to formation of substituted aldamine between the enzyme-bound pyridoxal-P and the compound.

One of the interesting problems concerned with the diamine transamination is

Table X. Stoichiometry of the Diamine Transamination

(A) 1, 2-Diaminoethane

Incubation time (min)	1, 2-Diaminoethane (decreased)	Pyruvate (decreased)	L-Alanine (increased)
		μmoles	
0	0	0	0
20	0.3	0.2	0.2
40	0.5	0.4	0.3
60	0.5	0.6	0.4

(B) Putrescine

Incubation time (min)	Putrescine (decreased)	Pyruvate (decreased)	L-Alanine (increased)
		μmoles	
0	0	0	0
20	1.0	1.0	1.2
40	1.9	1.7	1.8
60	2.4	2.5	2.2

(C) Cadaverine

Incubation time (min)	Cadaverine (decreased)	Pyruvate (decreased)	L-Alanine (increased)
		μmoles	
0	0	0	0
20	0.4	0.5	0.5
40	0.8	0.8	0.8
60	1.7	1.2	1.2

whether one or two amino groups of diamine are transfered. To clarify this question, the stoichiometry of the transamination using 1, 2-diaminoethane, putrescine and cadaverine was examined. As shown in Table X, only one amino group of the diamines tested is transfered to pyruvate to form alanine throughout the reaction time. It is conceivable that the only one amino group transfer from putrescine and cadaverine is due to a cyclization of the monoamine-products, γ-aminobutylaldehyde and δ-aminovaleraldehyde, respectively. The formation of Δ^1-pyrroline and Δ^1-pyperideine, a cyclized products of γ-aminobutylaldehyde and δ-aminovaleraldehyde, respectively, was shown by the reaction with *o*-aminobenzaldehyde. For 1, 2-diaminoethane the cyclization mechanism is ruled out, and one amino group transfer from the diamine may be ascribed to the presence of aldehyde group in monoamine-product interacting with other amino group to protect it against the enzymatic attack, or interfering with binding itself to the active site.

It is interesting to compare substrate specificity of two enzymes involved in the transamination of ω-amino acid, *i.e.* ω-amino acid: pyruvate and γ-aminobutyrate: α-ketoglutarate amino transferases. As discussed above, ω-amino acid : pyruvate aminotransferase catalyzes the transamination of various ω-amino acids other than β-alanine or γ-aminobutyrate, and pyruvate which is an exclusive amino acceptor. γ-Aminobutyrate transaminase, on the contrary, requires α-ketoglutarate as an amino acceptor and shows narrow substrate specificity (Table XI).[26] Although β-alanine is hardly transaminated by the bacterial enzyme, the mammalian enzyme reacts with the

Table XI. Amino donor specificity

Amino donor	Relative activity
γ-Aminobutyrate	100.0
δ-Aminovalerate	43.0
ε-Aminocaproate	9.8
7-Aminoheptanoate	1.7
Hypotaurine	3.6
L-Ornithine	2.8
D-Ornithine	5.4
L-Lysine	0.2
D-Lysine	1.7
Putrescine	7.7
Cadaverine	8.1
n-Amylamine	10.5
L-Alanine	0.2

Inert: Glycine, β-alanine, 8-aminooctanoate, β-amino-isobutyrate, DL-β-amino-*n*-butyrate, *p*-aminobenzoate, L-carnosine, aminoethylhydrogensulfate, taurine, amino-methanesulfonate, 3-aminopropanesulfonate, thiotaurine, D-alanine, L-aspartate, L-cysteinesulfinate, L-serine, D-serine, L-α-aminobutyrate, D-α-aminobutyrate, L-thereonine, L-methionine, L-valine, L-phenylalanine, L-2, 4-diaminobutyrate, *n*-butylamine, and *n*-amylamine.

compound to the same extent that with γ-aminobutyrate (Table I). These results suggest that there are at least two alternative metabolic pathway in bacteria, one is specific for β-alanine and the other for γ-aminobutyrate, and that in mammalian tissue both ω-amino acids are metabolized by a single enzyme. In addition to the distribution and the molecular structure as shown before, these differences in substrate specificity of ω-amino acid transaminase between bacterial and mammalian enzymes seem to be interesting from the viewpoint of evolution of the metabolic pathway and the enzyme structure concerned with ω-amino acid.

ABSORPTION SPECTRA

The absorption of ω-amino acid : pyruvate aminotransferase is somewhat a typical for a pyridoxal-P dependent transaminase.[15] There is not distinct maximum in the 420 to 430 nm region but rather a shoulder at 400 nm with the peak absorbance at 345 nm (Fig. 7). Pyruvate addition had no effect on spectrum, though L-alanine

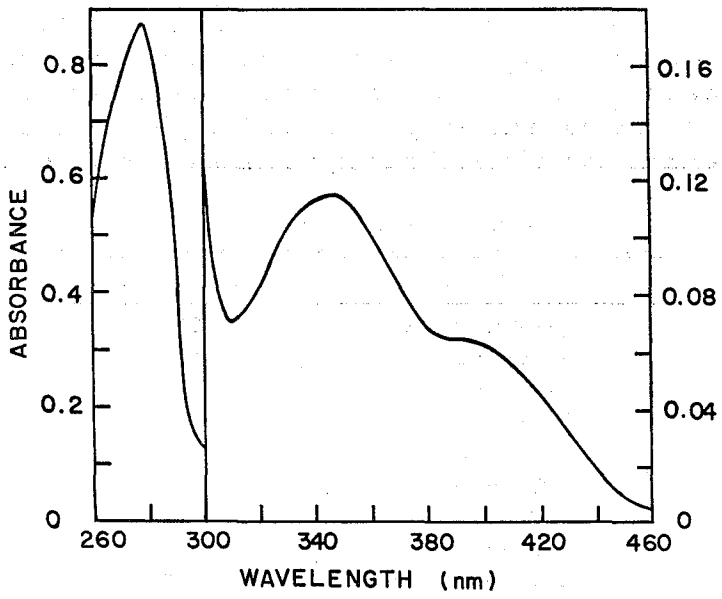


Fig. 7. Absorption spectrum of ω-amino acid: pyruvate aminotransferase.

caused a decrease in the 400 nm absorbance and an increase in the 345 nm absorbance. These results indicate that 400 nm absorbance is ascribed to a typical Schiff base formed between ε-amino group of the enzyme protein and pyridoxal-P and involved in the catalysis. These properties of the enzyme-bound cofactor have also been shown for L-lysine : α-ketoglutarate,[11] D-amino acid[29] and taurine : α-ketoglutarate aminotransferases.[13] The former two enzymes contain two pyridoxal-P per mole of enzyme showing an absorption maxima at around 340 nm and 415 nm. The 415 nm pyridoxal-P ascribed to a Schiff base formed between pyridoxal-P and enzyme protein is directly involved in the enzyme catalysis and can be resolved from the enzyme

protein to yield a catalytically inactive enzyme but still containing one mole of pyridoxal-P, "semiapoenzyme". Taurine transaminase exhibiting a similar absorption spectrum with that of ω-amino acid : pyruvate aminotransferase contains 4 moles of pyridoxal-P per mole of enzyme, of which one pyridoxal-P is important for the enzyme reaction. The case for ω-amino acid : pyruvate aminotransferase remain unlcear.

The absorption spectrum of the γ-aminobutyrate transaminase purified and crystallized from *Pseudomonas* sp. F–126 exhibits a maximum at 415 nm in the visible region and a low absorbance around 345 nm (Fig. 8).[26] This spectrum is quite different from that of ω-amino acid : pyruvate aminotransferase, though the two enzymes catalyze an analogous reaction and was obtained from the same organism. Spectrometric and protein chemical studies on the two enzymes are now progressing in our labolatory to clarify the discrepancy.

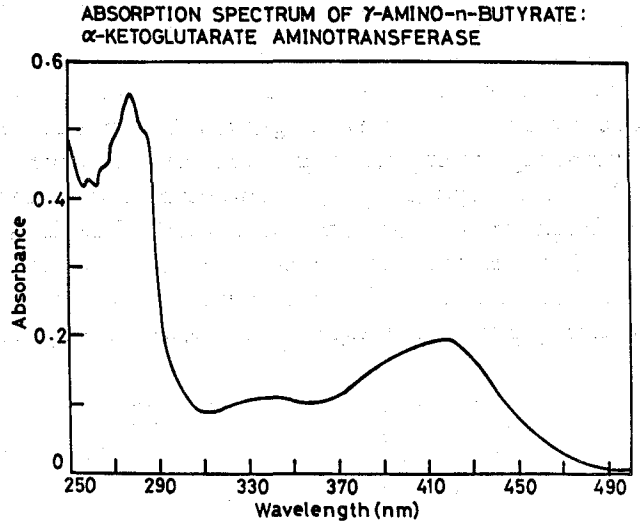
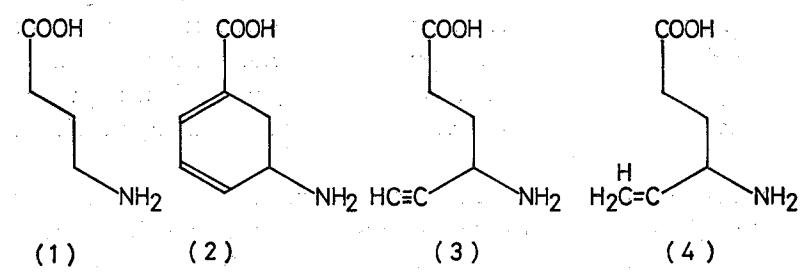


Fig. 8. Absorption spectrum of γ-aminobutyrate: α-ketoglutarate aminotransferase.

SUICIDE SUBSTRATE REACTION

A large number of compounds that are specific, irreversible enzyme inhibitor are found in natural products or have been synthesized.[30] These molecules are chemically unreactive themselves but can act substrate for the target enzyme. In the process of catalytic turnover, the enzyme become inactive. That is, the target enzyme catalyzes its own destruction. The fact that these molecules are chemically unreactive before turnover is the key to the specificity of these inhibitors. For this reason these compounds are referred to as a suicide substrate or a mechanism-based inactivator. For the suicide substrate of γ-aminobutyrate transaminase, several compounds have been successfully designed and tested, including gabaculine (5-amino-1, 3-cyclohexadienylcarboxylic acid) (2), γ-acetylenic-γ-aminobutyrate (3), γ-vinyl-γ-aminobutyrate (F). Rando and

Suicide substrate



Bangerter have shown that gabaculine specifically derivatizes the enzyme-bound pyridoxal-P after transamination by aromatizing to N-*m*-carboxyphenyl pyridoxamine-P (5) and inactivates a mouse brain γ-aminobutyrate transaminase.[31] When gabaculine was tested with ω-amino acid: pyruvate aminotransferase, a spectral change occurred, reminiscent of the effect of L-alanine on the spectrum (Fig. 9), and time-dependent inactivation of the enzyme ensued.[32] The inactivation followed pseudo-first order kinetics to complete loss of activity (Fig. 10). A plot of $1/K_{inact}$ *versus* 1/(gabaculine) yield a value of 36 min^{-1} for the limiting inactivation rate constant. Addition of amino donor to incubations protected the enzyme against gabaculine-induced inactivation, presumably by converting the enzyme to the nonsusceptible pyridoxamine-P form. Amino donor and pyruvate together, allowing conversion of pyridoxamine-P form to pyridoxal-P form, did not protect against the inactivation. The Ki value for gabaculine is 2.02 mM. The formation of N-*m*-carboxyphenylpyridoxamine-P (5) was confirmed by a heat treatment of the inactivated enzyme, followed by centrifugation to remove denatured protein. The supernatant showed the fluorescence spectra

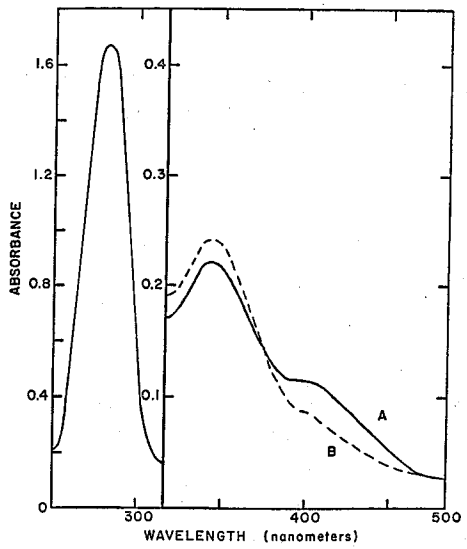


Fig. 9. UV-visible spectrum of ω-amino acid: pyruvate aminotransferase.
A; native enzyme, B; gabaculine-inactivated enzyme

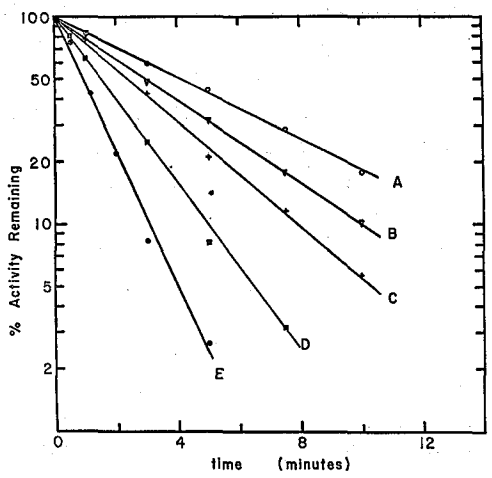
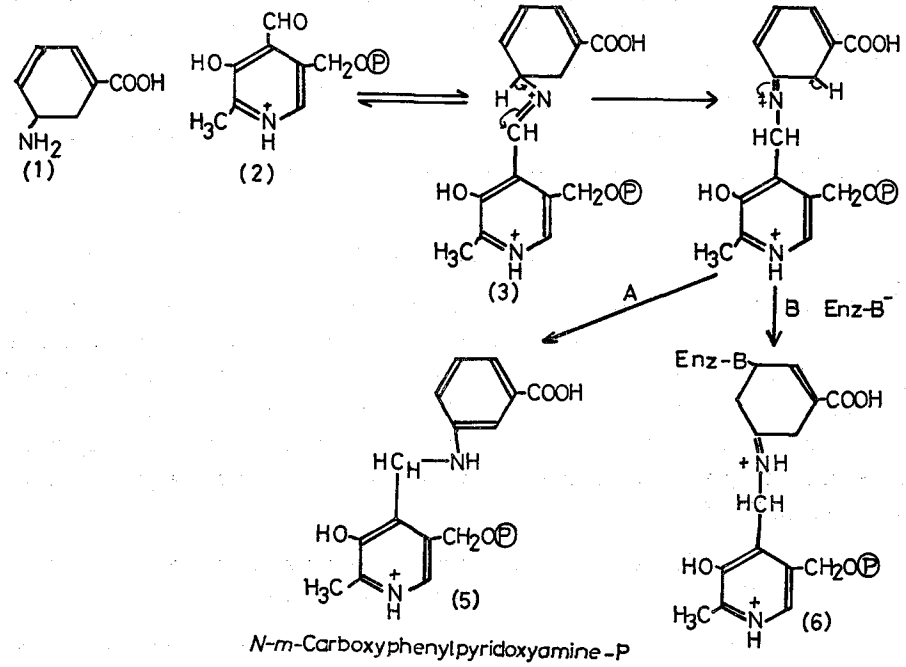


Fig. 10. *Inactivation of ω-amino acid : pyruvate aminotransferase by several fixed concentrations of* gabaculine.
A: 10.0 μmol, B: 12.5 μmol, C: 16.7 μmol, D: 25.0 μmol, E: 50.0 μmol

(excitation max=308 nm, emission max=408 nm) as those reported by Rando and Bangerter for authentic compound.[33] The fluorescent also migrated on thin layer chromatography in BuOH: H_2O: Acetate (2: 2: 1) and in paper electrophoresis in pyrimidine: acetate buffer (pH 3.5) with authentic N-*m*-carboxyphenylpyridoxamine-P (5). These results can be explained by the following mechanism.

Gabaculine inactivation mechanism



The initial step in the inactivation process lead to the rate limiting formation of the enzyme bound, transaminated product (4) *via* a Schiff base (3) formed between gabaculine (1) and pyridoxal-P (2). There are two alternative pathway followed. Sequence A would involve the simple aromatization of (4) to yield the *m*-anthranilic acid derivative (5), which remains enzyme bound, and the enzyme no longer show a catalytic activity any more. By heating the enzyme protein is denatured to release the compound. This mechanism would best accormodate the data, as shown for the γ-aminobutyrate transaminase from *P. fluorescence.*[34] The other mechanism, sequence B, would involve a Michael addition of an active site nucleophile to the complex (4) to generate the irreversibly inhibited enzyme (6). This mode of inhibition has precedent in the irreversible inactivation of pyridoxal-P linked aspartate transaminase by β, γ-unsaturated amino acids.[30] Stoichiometry of the inactivation showed that one mole of gabaculine bound with one mole of enzyme (tetramer), suggesting that the enzyme has one active site per tetramer. In order to explain this abnormality in the stoichiometry, one possible interpretation is that not all of the subunits have functional active sites. Other possibility is that the subunits interact strongly in negatively cooperative manner to produce a "quarter site reactivity". There is precedent for "half-site reactivity" on inactivation with alkylating agents, *e.g.* rat liver γ-cystathionase[35] and bacterial methionine-γ-lyase[36] on inactivation with the suicide substrate propagylglycine. Further study will clarify which case is applicable for ω-amino acid : pyruvate aminotransferase.

γ-Acetylenic-γ-aminobutyrate (3) and γ-vinyl-γ-aminobutyrate (4) have been demonstrated to be suicide substrates for γ-aminobutyrate transaminases from mammalian and *Pseudomonas.*[30] However, neither of these compounds functioned as substrate, nor did they induce detectable time- or concentration-dependent inactivation of ω-amino acid: pyruvate aminotransferase. Rather, γ-acetylenic-γ-aminobutyrate and γ-vinyl-γ-aminobutyrate behave merely as reversible competitive inhibitors with Ki values of 0.1 mM and 0.8 mM, respectively. Apparently, in this case, the unsaturated substituents misorient the analogues or distort the active site to prevent catalytic processing (and suicide reaction) from occurring.

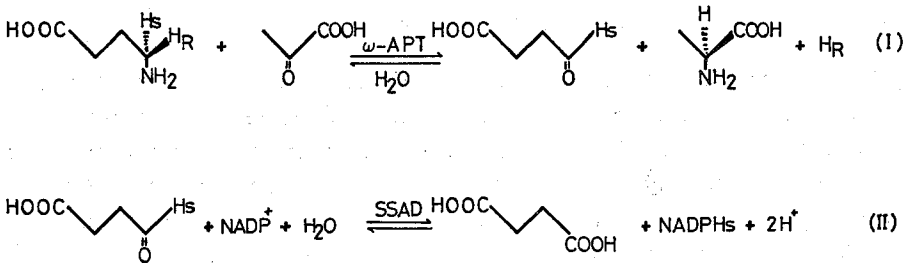
STEREOCHEMISTRY

It is interesting to know which hydrogen atom on the carbon carrying amino group of ω-amino acids is stereoselectively abstracted during the transamination. The stereochemistry of ω-amino acid : pyruvate and γ-aminobutyrate : α-ketoglutarate aminotransferases is examined using 4-*R*-(^{3}H)- and 4-*S*-(^{3}H)-γ-aminobutyrate as the amino donor.[37] The experiments were performed according to equation (I) and (II). When γ-aminobutyrate transaminase was employed, pyruvate was replaced by α-ketoglutarate in Eq. (I).

ω-APT: ω-Amino acid : pyruvate aminotransferase
SSAD : Succinic semialdehyde dehydrogenase

On chiral oxidation of γ-aminobutyrate, one of the two prochiral hydrogens at C–4 will

Stereochemistry



be exchanged into water (or some fraction may be transferred to the amino acceptor) and the other will remain in the product succinic semialdehyde (Eq. I). To trap this latter hydrogen in a stable and readily isolatable form, the transamination was coupled to the NAD-dependent succinic semialdehyde dehydrogenase to achieve direct transfer of the aldehyde to C–4 of NADH (Eq. 2). As shown in Table XII ω-amino acid: pyruvate aminotransferase has a clear preference for removal of the pro-*R* hydrogen

Table XII. Stereochemical Selectivity in γ-Aminobutyrate Transamination

Enzyme	Substrate	Specific activity /nCi μmol^{-1}	Product	μmol formed	Specific activity /nCi μmol^{-1} Observed	Predicted
ω-Amino acid: pyruvate aminotransferase	4-R-[^{3}H]GABA*	39.4	NADPH	2.50	5.89	0.00
			H_2O	2.50	19.0	<39.4
	4-S-[^{3}H]GABA	65.9	NADPH	2.75	66.1	65.9
			H_2O	2.75	9.19	0.00
GABA-T(α-oxoglutarate requiring)	4-R-[^{3}H]GABA	39.4	NADPH	2.50	36.7	39.4
			H_2O	2.50	11.6	0.00
	4-S-[^{3}H]GABA	65.9	NADPH	2.50	5.52	0.00
			H_2O	2.50	48.2	<65.9

* GABA: γ-Aminobutyrate

at C–4 of γ-aminobutyrate during oxidative deamination, while pro-*S* hydrogen remains bound to C–4 in succinic semialdehyde, available for subsequent hydride to NADP. This stereoselectivity corresponds to that experienced by glycine when it is processed by L-α-amino acid aminotransferases.[38] On the other hand, it may be seen in the table that γ-aminobutyrate transaminase exhibits the opposite stereochemical preference. This is in agreement with the recent observations that a mammalian γ-aminobutyrate transaminase also involved the abstraction of pro-*S*-hydrogen on the C–4 of γ-aminobutyrate[39] and that the *S*-isomer of γ-acetylenic-γ-aminobutyrate inactivated both bacterial and mammalian transaminases.[30] An *R*, *S* mixture of the compound, however, did not inactivate the ω-amino acid: pyruvate aminotransferase as described before. It may be that these opposing stereochemical preferences reflect differing modes of binding of γ-aminobutyrate in the active site of the two enzyme.

X-RAY CRYSTALLOGRAPHIC STUDIES

Over the past three years, X-ray crystallographic studies have been developed on aspartate : α-ketoglutarate aminotransferase from different enzyme source; pig heart cytoplasmic,[40] chicken heart mitochondrial[41] and cytoplasmic enzyme.[42] For the chicken heart mitochondrial enzyme the three dimensional structure at 4.5 Å resolution has been reported, elucidating the spatial location of subunits and active site. To clarify the unique properties of ω-amino acid: pyruvate aminotransferase discussed before and to compare with the mammalian transaminases crystallographic studies on the ω-amino acid transaminase have been initiated.[43] Single crystals ($2.0 \times 0.4 \times 0.4$ mm) of the enzyme were prepared by dialysis of the enzyme solution against ammonium sulfate solution (Fig. 11). An X-ray diffraction pattern at 2.4 Å resolution is presented in Fig. 12. Apparently little change in the diffraction pattern was observed after more than 40 hours of X-ray exposure. A typical precession photograph with the X-ray beam parallel to the long axis of the crystal gave the hk0 zone with *mm* symmetry. The upper levels also possessed *mm* symmetry. Since extinctions are observed for h+k+1 odd for general hkl reflections, the crystals are orthorhombic and belong to space group $I2_12_12_1$. The cell dimensions are a=124.1 Å, b=137.9 Å, and c=61.2 Å, and unit cell contains one subunit of the tetrameric enzyme.

In the case of the aspartate transaminase, several organic mercurials were bound with sulf hydryl groups to form good isomorphous derivatives. The ω-amino acid transaminase crystals could not form any derivative with mercurials, corresponding to the absence of reactive sulf hydryl groups in the native enzyme. On the other hand, potassium chloroplatinate gave the appropriate intensity changes in diffractions patterns. A search for other heavy-atom derivatives is now under way to determine the structure of the enzyme.

APPLICATIONS

The enzyme was successfully applied for the determination of L-alanine both in rate assay and endpoint assay systems (Fig. 13).[44] The principle of the assay relied on Eqs. (1) and (2).

$$\text{L-Alanine} + \text{Sulfoacetaldehyde} \overset{\omega\text{-APT}}{\rightleftharpoons} \text{Pyruvate} + \text{Taurine} \quad (1)$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{L-Lactate} + \text{NAD}^+ \quad (2)$$

ω-APT: ω-amino acid : pyruvate aminotransferase
LDH : L-lactate dehydrogenase

The decrease in absorbance at 340 nm of NADH is measured. Although the reaction (1) is reversible, L-alanine is virtually quantitatively converted to pyruvate by use of an excess of sulfoacetaldehyde. Conversion of pyruvate to lactate with lactate dehydrogenase also forces the equilibrium to the right. A quantitative relation between the absorbance change and the amount of L-alanine added was observed in the end-

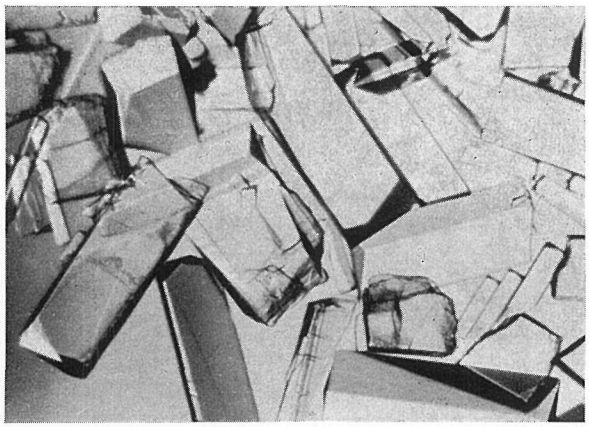


Fig. 11. Single crystals of ω-amino acid : pyruvate aminotransferase.

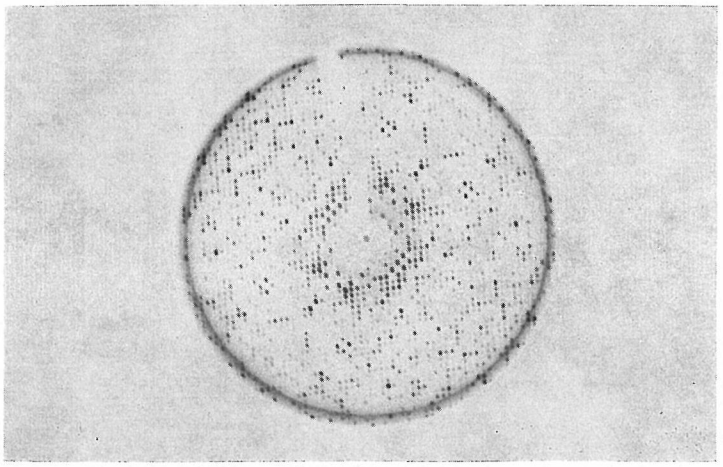


Fig. 12. X-ray diffraction pattern.

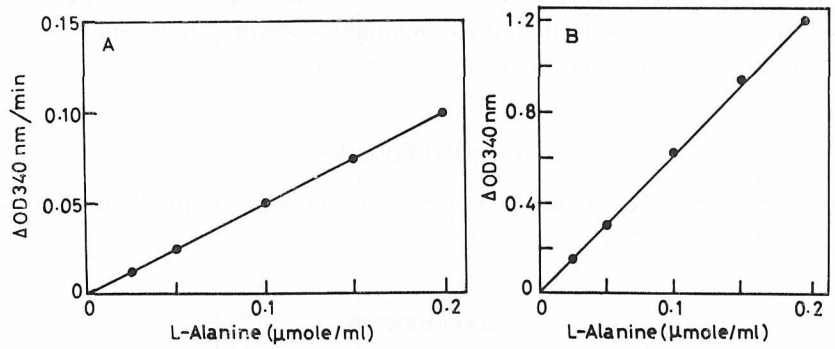


Fig. 13. Calibration curves for L-alanine in the rate assay (A) and the endopoint assay (B) systems.

point assay system, indicating that reactions (1) and (2) are virtually irreversible.

As mentioned above, the enzyme showed broad substrate specificity of ω-amino acids, and amines, but high specificity for α-amino acids, specific for L-alanine, reflecting the high specificity for amino acceptor, *i.e.* α-keto acids other than pyruvate are inactive as amino acceptors. This substrate specificity allows the assay to be specific for L-alanine. Substrate ω-amino acid, however, interfered competitively with the assay in the rate assay system, and α, ω-diaminocarboxylic acid, *i.e.* L-lysine and L-ornithine, also acted as a competitive inhibitor to a lesser extent (Table XIII). In the endpoint assay system a successful determination was carried out with longer incubation time and more enzyme, even if these compounds were present. The other non-substrate α-amino acids did not affect on the assay.

Table XIII. Effect of Amino Acids on the Assay

Amino acid	Amount (μmol)	Inhibition (%)
L-Ornithine	10	40
	1	0
L-Lysine	10	13
	1	0
β-Alanine	10	73
	1	32
	0.5	0
γ-Aminobutyrate	10	58
	1	0

No Effect: D-lysine, D-ornithine, D-alanine, L-aspartate, L- and D-glutamate, L- and D-α-aminobutyrate, L-phenylalanine, L-tryptophan, L-isoleucine, L-leucine, and L-valine.

Although we described here the application of the enzyme for the determination of L-alanine, the enzyme may be applied for the analysis of pyruvate, ω-amino acid and amines including their deaminated product. Considering the significant role of these compounds in a physiological function, the enzyme will also be expected to be of clinical value. Stereochemically labeled ω-amino acids or amines may be prepared by this enzyme. Studies along these lines are now in progress.

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